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Development of a new ARCHITECT automated periostin immunoassay

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ABSTRACT

Background: Periostin is being investigated as a potential biomarker for T-helper-2 (Th2)-driven asthma or eosinophilic inflammation and may help to identify patients more likely to benefit from interleukin-13-targeted treatments. We report the development and analytic performance of the investigational use only ARCHITECT Periostin Immunoassay, a new automated assay developed to detect serum periostin concentrations.
Methods: We assessed assay performance in terms of precision, sensitivity, linearity, interference from classical immunoassay interferents and representatives of common asthma medications, specimen handling, and isoform reactivity. The assay was also used to assess the biological variability of serum periostin concentrations in samples from healthy volunteers and from subjects with uncontrolled asthma (the intended use population).
Results: The percentage CVs for 5-day total precision, assessed using two instruments, was <6% across 2 controls and one serum-based panel. Limit of quantitation was 4 ng/mL (dilution adjusted concentration), suiting the needs for this application. Dilution analysis yielded linear results and no endogenous sample or drug interferences were observed. All known periostin isoforms expressed in the mature human lung were detected by the assay.
Conclusion: Our studies provide support that the ARCHITECT Periostin Immunoassay is a reliable and robust test for measuring serum periostin concentrations.

1. Introduction

Periostin is an 836-amino acid, approximately 90 kDa extracellular matrix protein encoded by the *POSTN* gene and expressed in a diverse range of adult and fetal tissues including lung, cardiac valve, adrenal gland, and thyroid [1,2]. Many differentially spliced isoforms of periostin exist and five post-natal human periostin isoforms with available sequences have been reported [3–5]. It appears to be involved in a variety of biological processes, such as tissue remodeling, cell proliferation, and angiogenesis, and has been reported to have a beneficial role in the development of cardiac valves, the development and repair of bone and teeth, and in healing after myocardial and vascular injury [2,6]. Periostin has also been implicated in a range of diseases including cancer, scleroderma, atopic dermatitis, proliferative diabetic retinopathy, and respiratory conditions such as idiopathic pulmonary disease, allergic rhinitis, and asthma [1,2,6]. Indeed, there is increasing evidence that periostin plays a key role in airway inflammation in patients with T-helper-2 (Th2)-driven asthma or eosinophilic inflammation [2,6,7].

In patients with Th2-driven asthma or eosinophilic inflammation, Th2 cells release the inflammatory cytokines interleukin (IL)-4 and IL-13, which induce periostin expression [1,7]. Periostin may contribute to eosinophil-mediated inflammation and fibrosis by enabling eosinophil infiltration at sites of airway inflammation and stimulating eosinophil adhesion and motility through actions on IL-13 and IL-5 [6]. Periostin gene expression is increased in airway epithelial and sputum cells of subjects with asthma compared with healthy control subjects [8,9]. Furthermore, when compared with fraction of exhaled nitric oxide, serum immunoglobulin (Ig) E concentrations and blood

Abbreviations: BGG, bovine gamma globulin; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; CV, coefficient of variation; HAMA, human *anti*-mouse antibodies; Ig, immunoglobulin; IL, interleukin; IPF, idiopathic pulmonary fibrosis; IUO, Investigational Use Only; kDa, kilodalton; LOQ, limit of quantitation; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; PAGE, polyacrylamide gel electrophoresis; PEI, polyethylenimine; PVDF, polyvinylidene difluoride; RF, rheumatoid factor; SD, standard deviation; SDS, sodium dodecyl sulfate; SRT, serum tube-red top; SST, serum separator tube; TBS, tris-buffered saline; Th2, T-helper-2.

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eosinophil count, serum periostin concentration has been reported to be the most significant single predictor of airway eosinophilia in patients with moderate to severe asthma who are symptomatic despite inhaled corticosteroid treatment [10]. Consequently, periostin has been identified as a potential biomarker for patients with Th2-driven asthma or eosinophilic inflammation [1,7,10–12] and, specifically, as a surrogate biomarker for the up-regulation of IL-13 [13].

Identifying blood-based biomarkers for IL-13 activity is important as treatments targeting the IL-13 pathway become available and identification of patients likely to be particularly responsive to IL-13-targeted therapy is required [12–15]. Collecting sputum samples to assess eosinophil inflammation is time-consuming and labor-intensive, making it impractical in routine clinical practice [12]. Furthermore, using serum IL-13 itself as a biomarker is challenging because it is expressed locally in inflamed tissue, resulting in very low concentrations in serum [12,16] (concentrations of serum IL-13 appear to be similar between healthy controls and subjects with asthma [16]). In contrast, periostin is found in much greater concentrations in serum and is therefore more readily measurable by immunoassay [12]. Measurement of serum periostin concentration may therefore help identify patients who would benefit from IL-13-targeted therapy. Indeed, patients with elevated periostin have been reported to show enhanced response to IL-13-targeted treatments such as tralokinumab [13] and lebrikizumab [17,18].

Here, we describe the development of the ARCHITECT Periostin Immunoassay, a new Investigational Use Only (IUO) immunoassay to determine serum periostin concentrations. The assay is currently being used to assess the utility of periostin as a biomarker in 2 Phase III studies (NCT02161757, NCT02194699) [15] investigating tralokinumab, an *anti*-IL-13 monoclonal antibody (mAb) in subjects with asthma [19,20]. We report the analytical performance of the assay in terms of precision, sensitivity, linearity, endogenous and drug interferents, specimen handling, and periostin isoform reactivity. We also provide data on the biological variability of periostin concentrations in samples from healthy volunteers and from subjects with uncontrolled asthma.

2. Materials and methods

2.1. Assay description

The IUO ARCHITECT Periostin Immunoassay has been developed for use with the ARCHITECT Immunoassay *i* System (Abbott Laboratories, Abbott Park, IL) [21].

It is a mAb sandwich two-step immunoassay for the quantitative determination of periostin in human serum using chemiluminescent magnetic immunoassay technology. Periostin, the analyte, is captured by microparticles coated with an *anti*-periostin mAb and subsequently detected with a second *anti*-periostin mAb conjugated with acridinium (Fig. 1). Numerous antibody pairings were initially evaluated for the potential to address key performance measures before selection of the final mAb pairing for the assay.

In the first step of the immunoassay process, a reaction mixture consisting of 40 µL of ARCHITECT system line diluent, 10 µL of specimen, and 50 µL of microparticles coated with an anti-periostin mAb is vortexed and incubated for 18 min (Fig. 1). The paramagnetic beads are captured and washed with diluent, which eliminates any remaining sample, and 50 µL of anti-periostin mAb conjugate labeled with acridinium is added. After mixing by vortex and a 4-min incubation, the paramagnetic particles are captured again and unbound acridinium conjugate is removed in a diluent wash. Pre-trigger and trigger solutions are added, resulting in a chemiluminescent signal reportable as relative light units, which are directly correlated to the amount of periostin present (Fig. 2). The assay is fully automated, and the ARCHITECT *i* System has a throughput of 200 tests per hour [21].

The assay was standardized early in the development process using gravimetrically prepared periostin isoform 1 with protein concentration determined using an extinction coefficient established by amino acid analysis. The assay calibration range (0–100 ng/mL; Fig. 2) was selected based on 447 samples taken from a Phase IIb study (NCT01402986) [13] of subjects with severe, uncontrolled asthma, with a pre-treatment periostin concentration range of 5–73 ng/mL.

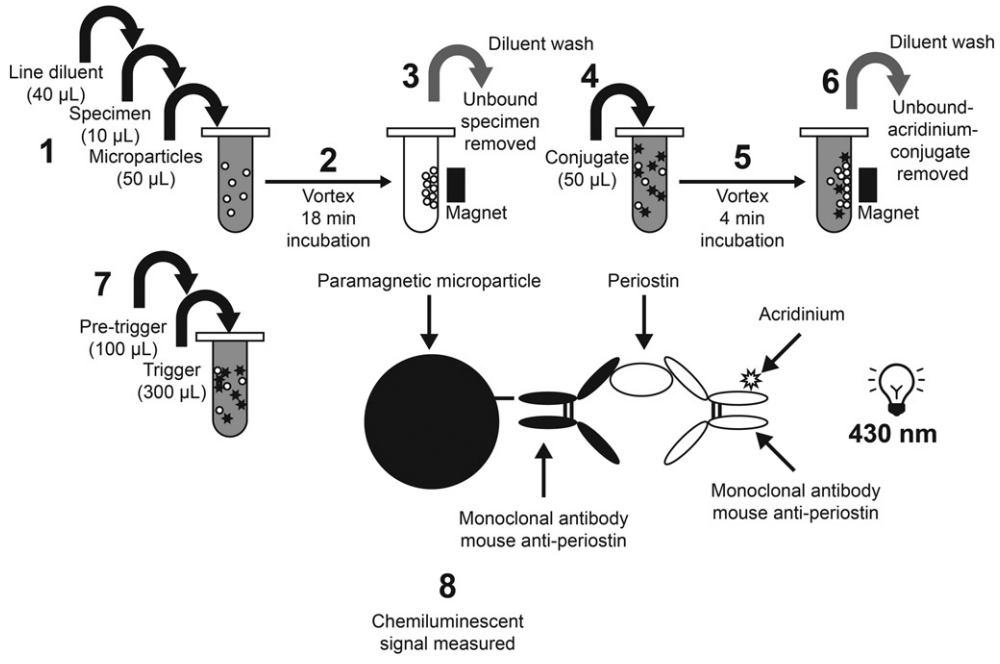

Fig. 1. The ARCHITECT Periostin Immunoassay method.

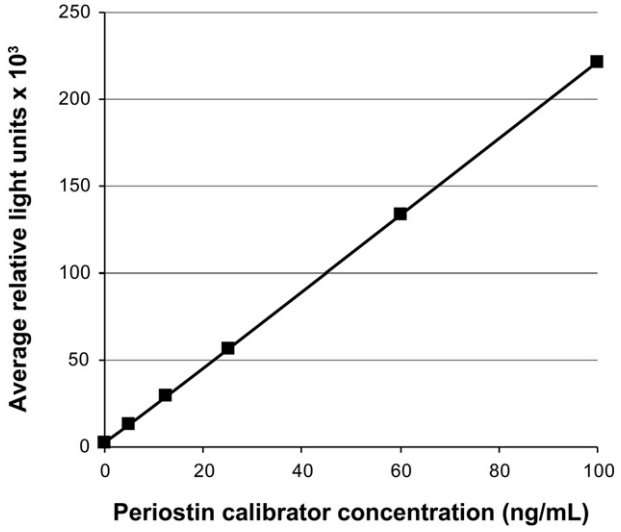

Fig. 2. Relationship (simple linear regression analysis) between relative light units and periostin concentration: 6-point periostin calibration curve.

2.2. Assay performance

2.2.1. Samples

Normal samples from healthy volunteers were obtained from ProMedDx, LLC. Samples from subjects with idiopathic pulmonary fibrosis (IPF) were obtained from Bioreclamation IVT (New York, NY). Samples from subjects with asthma used in periostin concentration assessments were obtained from a Phase IIb study (NCT01238861) [22]. For the specimen handling procedures, samples from subjects with asthma were obtained with written consent from volunteers at Abbott Laboratories (Abbott Park, IL). All samples were collected in line with approved protocols from institutional review boards.

2.2.2. Precision testing

Assay precision was evaluated according to the Clinical Laboratory Standards Institute (CLSI) EP05-A2 guidelines [23], with the exception that the replicates were evaluated across 5 days instead of 20 days. Two levels of control and 1 normal human serum panel were run in replicates of 4, twice daily with a 2 h or greater gap between runs. Panels were run for 5 days on 2 different ARCHITECT *i*2000 instruments. For controls, recombinant periostin was spiked into an artificial matrix (phosphate buffered saline, pH 7.4, protein stabilizers, and antimicrobial) to concentrations of 8 ng/mL (low concentration control) and 30 ng/mL (high concentration control). The human serum panel represents a native or endogenous periostin sample.

2.2.3. Dilution linearity and spike recovery

Dilution linearity was assessed according to the CLSI guideline EP06-A [24]. A sample of human serum with a high periostin concentration (from a subject with IPF) was diluted serially from 400 ng/mL to 0.4 ng/mL with human serum that was stripped of periostin using paramagnetic microparticles coated with an *anti*-periostin mAb. Regression analysis was used to compare expected and observed periostin concentrations. For spike recovery, seven normal serum specimens were spiked to 20, 50, and 100 ng/mL with recombinant periostin and assessed for recovery [25].

2.2.4. Interferents

The potential for interference from hemoglobin (>200 mg/dL), protein (>12 g/dL), bilirubin (>20 mg/dL), and triglyceride (>3000 mg/dL) was tested using hemolysate, bovine gamma globulin (BGG), conjugated and unconjugated bilirubin, and intralipid triglyceride, respectively. The interference was designed with the aim of achieving a mean percentage difference of ≤10% between periostin concentrations with and without interferent. Tests were performed according to the CLSI EP07-A2 guidelines [26]. Hemoglobin, conjugated and unconjugated bilirubin, protein, and triglycerides were spiked into normal human serum (to create one sample for each) and their presence was confirmed on a clinical chemistry instrument (ARCHITECT c8000; Abbott Laboratories, Park, IL) prior to comparison with unspiked controls. Spiking agents did not exceed 5% of the total mixture volume.

The potential for interference from human *anti*-mouse antibodies (HAMA), rheumatoid factor (RF), and heterophile antibodies was tested in 16 HAMA samples, 25 RF samples, and 19 heterophilic samples with and without 1 mg/mL blocking agent (mouse IgG). Linear regression was used to compare periostin concentration (ng/mL) with and without the blocking agent.

Cross-reactivity with selected representative common asthma medications was assessed using normal human serum spiked with medications at the following concentrations: salbutamol (drug concentration tested: 1.67 μmol/L), fluticasone (345 pg/mL), prednisone (0.82 μmol/L), desloratidine (0.97 μmol/L), montelukast (0.9 μmol/L), ipratropium bromide (300 pg/mL), cromolyn sodium (0.03 μg/mL), and theophylline (222 μmol/L). These concentrations were based on CLSI guideline EP07-A2 [26], or were at least three times the greatest concentration reported following therapeutic dosage or the greatest expected concentration (if known). If the expected blood concentration was not known, it was assumed that the therapeutic dose was distributed in 5 L of blood, and a concentration of at least three times this amount was used.

2.2.5. Limit of quantitation (LOQ)

LOQ was evaluated according to CLSI guideline EP17-A2 [27]. LOQ panels were prepared in goat serum, which was selected because it does not have any reactive components that yield signal in the assay. Goat serum was used to dilute standard human serum to create periostin panel levels at 0.5, 1.0, 2.0, 3.0, 3.8, 5.0, 6.2, 7.0, and 8.0 ng/mL. Each level was tested in five runs on each of two different instruments with two reagent lots over 3 days in replicates of 10 each.

2.3. Characterization of assay antibodies

Purified human periostin full-length and N-terminal segment proteins were used to assess antibody binding. Inspection of four human periostin isoforms showed that the first 669 amino acids are identical. The mature common N terminal fragment was thus cloned from the N at position 22 to the Y at position 669 such that the native signal peptide was replaced with the CD33 leader and a C terminal 6 X His tag was added to aid purification.

2.3.1. Electrophoretic and immunoblotting studies

Purified human periostin full-length and N-terminal segment proteins were electrophoresed in non-reduced form on 4–20% Bio-Rad Criterion Tris-HCl sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels using standard Tris/Glycine/SDS running buffer at a total protein load of 0.25 μg per gel lane. Gel migration was standardized using BioRad Precision Plus Dual Xtra Standards (catalog #161-0377). Following electrophoresis, the gels were stained for protein using Coomassie Brilliant Blue R-250 reagent or electrotransferred to a polyvinylidene difluoride (PVDF) membrane using a BioRad TransBlot Turbo instrument. For development of Western blots, the membranes were first blocked with Protein-Free T20 Blocking Buffer (Thermo Scientific catalog #37571), briefly washed with 3 exchanges of tris-buffered saline (TBS) buffer, and then probed with *anti*-periostin 4B4.B11 or 7B5.C4 monoclonal antibody in a 100 mL solution at a concentration of 1 μg/mL overnight (approximately 18 h). The probed membranes were then briefly washed with three exchanges of

TBS buffer and incubated with 100 mL goat *anti*-mouse IgG (heavy and light chains) alkaline phosphatase conjugate at a dilution of 1:1000 from the commercial reagent solution (BioRad catalog #170-6520) for 3 h. The membranes were then again briefly washed with three exchanges of TBS buffer. Development of the blots was carried out using an alkaline phosphatase substrate development kit (BioRad catalog #170-6432). Digital images of protein-stained gels and Western immunoblots were acquired with a BioRad GS-800 Scanning Densitometer.

2.3.2. Testing for isoforms

For the isoform analyses, five periostin isoforms found in lung tissue (isoforms 2, 3, 4, 7, and 8) plus isoform 1 were expressed in human embryonic kidney cells and evaluated by SDS-PAGE, Western blot, and in the ARCHITECT Periostin Immunoassay. The DNA sequence for isoform 1 was obtained from GenBank accession number NM_006475.2. The sequences for isoforms 2, 3, 4, 7, and 8 were then determined from the literature [28]. The sequences were submitted to GenScript USA, Inc (Piscataway, NJ) and subjected to their algorithm for optimizing codon usage for maximum expression in human cells. The DNA was synthesized and inserted into the expression vector pcDNA3.1 + (Life Technologies, Grand Island, NY). The constructs were sequenced by the vendor, the sequences were translated using Vector NTI software (v11, Life Technologies), and aligned with the original protein sequences to verify that the codon-optimized sequences coded for identical amino acid sequences. The Vector NTI software was also used to calculate molecular weights for each isoform.

The human embryonic kidney cells (HEK-293-6E, National Research Council Canada) were propagated in suspension culture using FreeStyle 293 media (Life Technologies). Flasks were seeded with 0.5×10^6 cells/mL in 100 mL media and incubated for 2 days at 37 °C in 8% CO_2. The cells were then transfected with plasmid DNA using polyethylenimine (PEI; Polysciences, Inc., Warrington, PA) at a DNA:PEI ratio of 1:2.5 using 0.75 pg DNA per cell. At 4-h post-transfection, the cultures were fed with 5 g Tryptone N1 per liter of culture media (Organotechnie, La Courneuve, France). At 6 days post-transfection, the cultures were harvested and the media clarified by centrifugation. A flask of cells that was not transfected was harvested and clarified to use as a media control.

2.4. Specimen handling

Serum samples from 10 subjects with a history of asthma were collected to assess sample stability at room (30 °C) and refrigeration (2–8 °C) temperatures, when frozen at −10 °C and −70 °C, and following repeated freeze/thaw cycles.

2.4.1. Stability at room and refrigeration temperatures

All samples were stored at room temperature for 8 h, before being kept at 2–8 °C or 30 °C for 7 days. Both normal serum tube-red top (SRT) and serum separator tube (SST) types were used. Samples were analyzed 'on the clot' (serum is not poured out of the draw tube, to mimic 'worst-case' sample handling) and 'off the clot' (serum is poured or pipetted into a secondary container) to mimic potential variation in sample handling. Percentage difference in periostin concentration vs. baseline was calculated for each sample on days 1 (i.e., after 24 h), 4, and 7; it was necessary for the upper and lower 95% confidence intervals (CIs) around the mean percentage difference to be ≤10% to support storage at a given temperature.

2.4.2. Stability when frozen at −10 °C and −70 °C and freeze/thaw cycle stability

Samples were frozen at −10 °C and −70 °C for 35 days. All samples were evaluated 'off the clot'. Both SRT and SST types were used. Percentage difference in periostin vs. baseline (room temperature) was calculated for each sample on days 7, 14, 21, 28, and 35; the upper and lower 95% CI around the mean percentage difference needed to be ≤10% to support storage at a given temperature.

In the freeze/thaw cycles, specimens frozen at −70 °C for at least 12 h were transferred to room temperature for a minimum of 1 h, prior to testing. Up to 4 freeze/thaw cycles were conducted for both SRT and SST 'off the clot' specimens. Serum specimens were taken from 8 subjects with asthma.

2.5. Periostin concentration assessments in healthy volunteers and asthma populations

Serum samples from apparently healthy male and female volunteers ($n = 757$) and from adult male and female subjects with uncontrolled asthma ($n = 585$) were evaluated using the ARCHITECT Periostin Immunoassay to provide information on the distribution of periostin concentrations in these populations. Data were evaluated descriptively and analyzed by gender, age, and ethnicity.

3. Results

3.1. Assay performance

3.1.1. Precision testing

Overall 5-day total precision, measured using percentage coefficient of variation (%CV), was <6% across 2 controls and 1 serum-based panel, and using (%CV) (Table 1).

3.1.2. Dilution linearity and spike recovery

Specimen dilution analysis yielded linear results across the dynamic range of the assay. The average correlation between observed and expected values was 0.99 (Fig. 3); residual plots were reviewed and no systematic biases were observed (data not shown). The mean (range) spike recovery was 104% (99–109%) for 20 ng/mL, 105% (99–113%) for 50 ng/mL, and 103% (100–105%) for 100 ng/mL periostin concentrations.

3.1.3. Interferents

Interference was <10% for protein, bilirubin, triglycerides, and hemoglobin (Table 2), and no interference was observed for HAMA, RF, and heterophilic antibody samples in the presence or absence of blockers (Fig. 4); residual plots were reviewed and no systematic biases were observed (data not shown). No drug interference was observed; the mean difference in periostin concentration when tested with or without key asthma medications ranged from −2.1% to 1.8% (data not shown).

Table 1
Five-day assay precision evaluation summary.

			%CV (SD)				
Level	*N*	Mean periostin concentration (ng/mL)	Within run	Between run	Between day	Between instrument	Overall
Low concentration control	80	8.0	4.0 (0.3)	1.0 (0.1)	0.0 (0.0)	4.0 (0.3)	5.7 (0.5)
High concentration control	80	29.6	2.3 (0.7)	1.5 (0.4)	0.0 (0.0)	0.0 (0.0)	2.8 (0.8)
Human serum panel	80	12.3	3.1 (0.4)	2.2 (0.3)	0.0 (0.0)	0.9 (0.1)	3.9 (0.5)

Abbreviations: %CV, percentage coefficient of variation; SD, standard deviation.

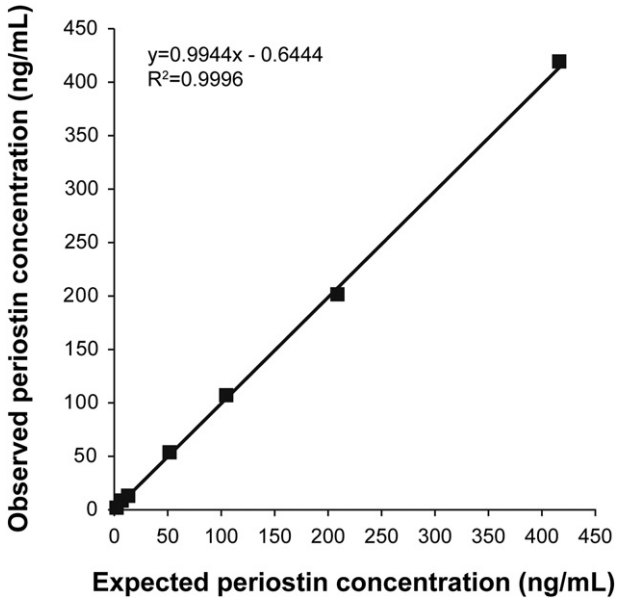

Fig. 3. Dilution linearity (simple linear regression analysis, $n=9$) of the ARCHITECT Periostin Immunoassay in an endogenous sample (IPF). Abbreviation: R^2, coefficient of determination.

3.1.4. LOQ

Nine serum periostin panel concentrations (ranging from 0.5 to 8.0 ng/mL) were used in determining the LOQ. The LOQ for these concentrations ranged from 1.0 to 3.8 ng/mL. Thus, overall, LOQ for the assay is approximately 4 ng/mL.

3.2. Characterization of assay antibodies

Western blot analyses showed that the immunoassay antibodies bind to the N-terminal fragment of periostin (common to all isoforms), and that the antibodies detect both monomeric periostin (at approximately 75 kDa), and dimeric and multimeric forms of periostin (at 150–250 kDa) (Fig. 5). In a normal human serum sample spiked to 50 ng/mL with the N-terminal fragment of recombinant periostin, recovery was 91%.

All known periostin isoforms expressed in the mature lung (2–4, 7, 8) are detected by the ARCHITECT Periostin Immunoassay (Table 3). The assay also detected lung isoform 1 (used as the calibrator antigen), which is present in fetal lung but not found in non-small-cell lung cancer (NSCLC) or adult lung tissue [29]. The calculated molecular weights for the expressed isoforms, without the signal sequence, were calculated (data not shown) and used to confirm expression of the intended isoforms.

Table 2
Interference testing with hemoglobin, protein, bilirubin, and triglyceride.

Interferent and test	Mean periostin concentration (ng/mL)		% difference
	Control	With interferent	
Hemoglobin			
Hemolysate			
>200 mg/dL total hemoglobin	20.1	19.0	-5.5
Protein			
BGG			
>12 g/dL total protein	11.6	12.1	4.3
Bilirubin			
Unconjugated			
>20 mg/dL total bilirubin	11.5	11.3	-1.7
Conjugated			
>20 mg/dL total bilirubin	14.7	16.0	8.8
Triglyceride			
Intralipid			
>3000 mg/dL triglyceride	11.0	10.8	-1.8

Abbreviation: BGG, bovine gamma globulin.

3.3. Specimen handling

3.3.1. Stability at room and refrigeration temperatures

The mean (95% CI) percentage difference from baseline in periostin concentrations measured after sample storage at room temperature (30 °C) in SST 'off the clot' ($n = 10$) was −0.8% (−2.5% to 0.8%) on day 1, −8.5% (−10.1% to −6.8%) on day 4, and −23.9% (−25.0% to −22.8%) on day 7. The equivalent values after sample storage at 2–8 °C in SST 'off the clot' ($n = 10$) were: −4.33% (−4.8% to −0.3%) on day 1, −24.99% (−17.1% to −2.3%) on day 4, and −33.26% (−23.9% to −11.0%) on day 7. Similar results were reported for SST samples tested 'on the clot' and SRT samples tested 'on' and 'off the clot' at room and refrigeration temperatures (data not shown), indicating sample stability at room temperature and 2–8 °C for 24 h.

3.3.2. Stability when frozen at −10 °C and −70 °C and freeze/thaw cycle stability

The mean (95% CI) percentage difference from baseline in periostin concentrations measured after sample storage frozen at −10 °C in SST 'off the clot' ($n = 10$) was −3.4% (−4.9% to −2.0%) on day 7, −2.3% (−3.7% to −0.8%) on day 14, 0.9% (−1.1% to 2.8%) on day 21, −1.2% (−2.9% to 0.6%) on day 28, and −3.1% (−5.0% to −1.2%) on day 35. The equivalent values after sample storage frozen at −70 °C in SST 'off the clot' ($n = 10$) were: -1.7% (−3.0% to −0.4%) on day 7, −2.5% (−5.2% to 0.1%) on day 14, 2.3% (0.5% to 4.1%) on day 21, −1.8% (−0.8% to −2.8%) on day 28, and −5.8% (−7.5% to −4.0%) on day 35. Similar results were reported for SRT samples tested 'off the clot' at −10 °C and −70 °C temperatures (data not shown), indicating sample stability at these temperatures for >1 month. For sample storage beyond 24 h of collection, freezing (≤ −10 °C) is therefore recommended. Samples were stable for up to 2 freeze/thaw cycles.

3.4. Periostin concentration assessments in healthy volunteers and asthma populations

Serum samples from 757 apparently healthy volunteers showed a mean periostin concentration of 16 ng/mL, with similar mean periostin concentrations regardless of gender or ethnicity (Table 4). Mean periostin concentration appeared to be slightly elevated in subjects aged 16–18 y (19 ng/mL, range 7–56 ng/mL; Table 4), and particularly in males in this age group (21 ng/mL, range 7–56 ng/mL, $n = 173$). Females in this age group had a mean periostin concentration of 17 ng/mL (range 7–33 ng/mL, $n = 187$).

Serum samples from 449 subjects with uncontrolled asthma appeared to show slightly greater mean periostin concentrations (19 ng/mL, range 5–73 ng/mL; Table 5) than those in the healthy volunteer population (periostin concentrations appear normally distributed). In subjects with uncontrolled asthma, mean periostin concentrations were broadly similar regardless of gender or ethnic group (Table 5).

4. Discussion

We showed that the IUO ARCHITECT Periostin Immunoassay is a reliable and robust assay, as indicated by: levels of precision of <6 %CV within run, between run, between day, and across 2 instruments; high sensitivity for periostin (i.e., low LOQ); and stability over 24 h at room and refrigeration temperature (beyond 24 h of collection, freezing at -10 °C or colder is recommended). Additionally, there was no notable interference from common asthma medications or classical immunoassay interferents, and good dilution linearity from 400 ng/mL to 0.4 ng/mL

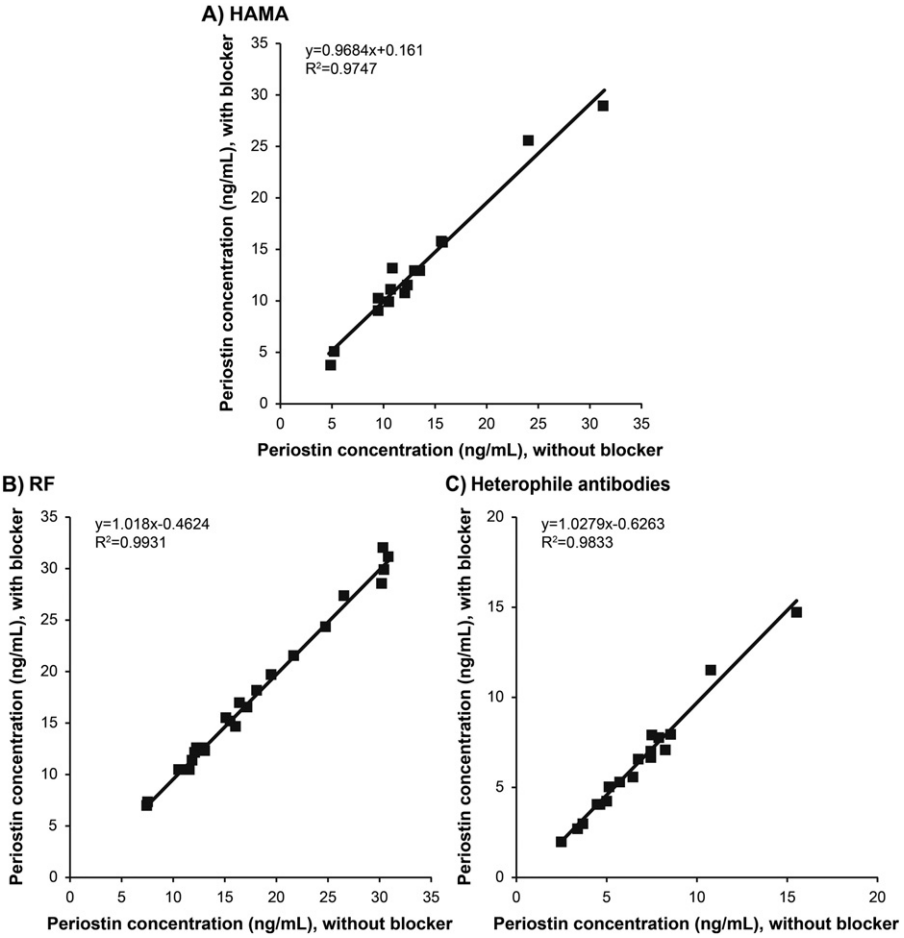

Fig. 4. Evaluation (simple linear regression analysis) of potential interference from HAMA (panel A, $n = 16$), RF (panel B, $n = 25$), and heterophile antibodies (panel C, $n = 19$). 1 mg/mL mouse IgG was used as blocker. Abbreviations: HAMA, human *anti*-mouse antibodies; RF, rheumatoid factor; R^2, coefficient of determination.

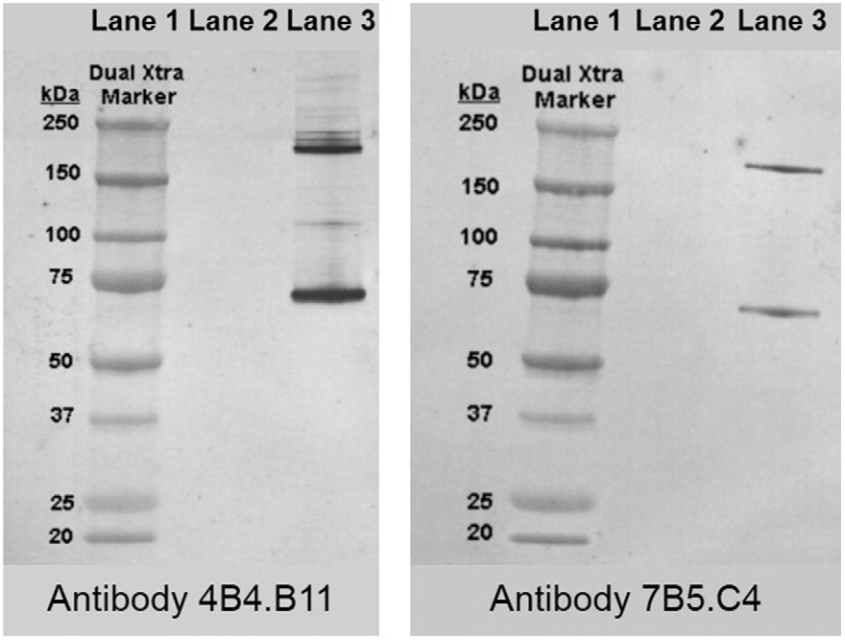

Fig. 5. Western blot analyses showing detection of monomeric (approximately 75 kDa), and dimeric and multimeric (150–250 kDa) periostin forms by the antibodies (4B4.B11 on left and 7B5.C4 on right) used in the ARCHITECT Periostin Immunoassay. Lane 1: Dual Xtra Molecular Weight Markers; Lane 2: Empty; Lane 3: N-terminal periostin fragment (non-reduced and not boiled).

Table 3
Periostin isoforms detected by the ARCHITECT Periostin Immunoassay.

Isoform	Fetal	NSCLC	Normal	Exons								Isoform concentration (ng/mL)
1	+	−	−	1–13	14–16	17	18	19	20	21	22–23	Calibrator antigen
2	+	+	+	1–13	14–16			19	20	21	22–23	6656
3	+	+	+	1–13	14–16		18	19	20		22–23	7720
4	+	+	+	1–13	14–16			19	20		22–23	9721
5	+	−	−	1–13	14–16	17	18	19	20		22–23	Not tested
6	−	−	−	1–13	14–16	17		19	20	21	22–23	Not tested
7	+	+	+	1–13	14–16				20	21	22–23	5338
8	+	+	+	1–13	14–16				20		22–23	3651

+/− indicate whether the periostin isoform is present (+) or absent (−) in the tissue type. Isoforms 5 and 6 were not tested as they are not present in normal lung tissue. Calibrator antigen concentration was assigned with an absorbance at 280 nm using an extinction coefficient established by amino acid analysis. Isoforms were diluted into the dynamic range of the assay and then the results were corrected for dilution factor. Abbreviation: NSCLC, non-small-cell lung cancer.

was observed, with an average correlation between observed and expected values of 0.99.

The ARCHITECT Periostin Immunoassay is a two-step assay in which periostin is captured by microparticles coated with an *anti*-periostin mAb prior to detection by a second, non-competing *anti*-periostin mAb conjugated with acridinium. This two-step assay approach reduces the likelihood of high-dose hook effects [21], thus minimizing the risk of false-negative results in samples high in periostin [30]. It also reduces assay non-specific binding and prevents the exposure of acridinium to potential interferents such as HAMA [21]. Furthermore, the assay contains blockers to target interferents such as HAMA, RF, and heterophile antibodies.

Our findings indicate that the antibodies used in the ARCHITECT Periostin Immunoassay bind to the N-terminal fragment of periostin and that they detect monomeric, dimeric, and multimeric forms of this analyte. The assay also detects all known periostin isoforms found in the lung, indicating a potential suitability for use in inflammatory respiratory conditions where periostin may be implicated. Indeed, the ARCHITECT Periostin Immunoassay is currently being used to assess the utility of periostin as a biomarker in two Phase III studies of tralokinumab (an *anti*-IL-13 mAb) in subjects with asthma (NCT02161757, NCT02194699) [15]. The assay calibration range in our studies was selected based on the range of periostin concentrations (5–73 ng/mL) in a Phase IIb study of subjects with severe, uncontrolled asthma. Additionally, we used the ARCHITECT Periostin Immunoassay to analyze additional serum samples taken from subjects with uncontrolled asthma from a second Phase IIb study and found periostin concentrations ranging from 8 to 104 ng/mL with a similar mean periostin concentration regardless of ethnic group or gender. Thus, the ARCHITECT Periostin Immunoassay should reliably detect the full range of periostin concentrations in subjects with asthma and be appropriate for use in this group regardless of age, gender or ethnicity.

As expected, our analysis of periostin concentration in serum samples appeared to show slightly elevated mean periostin concentrations in samples from subjects with uncontrolled asthma compared with apparently healthy volunteers. This is consistent with reports of elevated serum periostin concentration in subjects with asthma, particularly those with Th2-driven asthma or eosinophilic inflammation [10,31]. Furthermore, our analyses suggested slightly elevated mean periostin concentrations in young healthy volunteers (16–18 y) compared with volunteers aged ≥19 y. Again, this is consistent with reports of elevated periostin in children and young people compared with adults, regardless of asthma [32,33].

In addition to its role in asthma, periostin has also been implicated in allergic diseases such as atopic dermatitis, allergic rhinitis, and eosinophilic otitis media, and non-allergic diseases such as IPF, scleroderma, proliferative diabetic retinopathy, and bone marrow fibrosis [1,6]. It is also over-expressed in many cancers [6]. Thus, the potential utility of the ARCHITECT Periostin Immunoassay is far-reaching, although further research is required to assess the use of the immunoassay in other respiratory and non-respiratory conditions. In conclusion, our studies indicate that the IUO ARCHITECT Periostin Immunoassay is a reliable and robust test for the measurement of serum periostin concentrations.

Table 4
Distribution of periostin concentrations in samples from apparently healthy volunteers.

		Periostin concentration (ng/mL)		
	Number of samples	Mean	Median	Range
All	757	16	15	5–56
Gender				
Female	384	16	15	5–37
Male	373	17	15	5–56
Age, y				
16–18	360	19	18	7–56
19–21	55	14	16	9–32
22–30	53	15	14	5–30
31–40	91	13	13	5–37
41–50	92	13	13	5–27
51–60	64	13	12	7–24
61–72	42	14	13	8–26
Ethnicity				
African American/Black	192	16	15	5–56
Asian	165	17	16	5–41
Caucasian/White	200	16	14	6–43
Hispanic	200	17	16	6–36

Table 5
Distribution of periostin concentrations in samples from subjects with uncontrolled asthma.

		Periostin concentration (ng/mL)		
	Number of samples	Mean	Median	Range
All	449	19	16	5–73
Gender				
Female	296	19	17	5–73
Male	153	18	16	5–54
Age, y				
18–21	9	21	19	10–39
22–30	21	17	16	8–31
31–40	77	17	15	7–39
41–50	109	18	16	5–60
51–60	140	18	16	5–61
61–70	75	22	19	7–73
71–80	18	22	21	8–47
Ethnicity				
African American/Black	14	21	14	7–54
American Indian/Eskimo	27	18	17	8–40
Asian	158	22	19	7–73
Caucasian/White	174	16	15	5–45
Hispanic	76	18	16	6–61

Conflict of interest

N.M. Jeanblanc, P.M. Hemken, M.J. Datwyler, S.E. Brophy, and G.J. Davis are employees of Abbott Laboratories. T.S. Manetz, R. Lee, M. Liang, P. Chowdhury, R. Varkey, E.P. Grant, K. Streicher, L. Greenlees, and K. Ranade are employees of MedImmune. G.J. Davis, S.E. Brophy, and P.M. Hemken have stocks/options in Abbott Laboratories and AbbVie. T.S. Manetz, R. Lee, M. Liang, P. Chowdhury, R. Varkey, E.P. Grant, K. Streicher, L. Greenlees, and K. Ranade have stocks/options in AstraZeneca. P. Chowdhury has been a speaker, chairperson, and consultant for CHI. N.M. Jeanblanc, S.E. Brophy, R. Lee, M. Liang, P. Chowdhury, R. Varkey, E.P. Grant, K. Streicher, L. Greenlees, K. Ranade, and G.J. Davis are named inventors on a pending patent application (international patent application number PCT/US2015/01467, entitled 'Novel assay to detect human periostin') related to this manuscript.

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References

[1] K. Izuhara, K. Arima, S. Ohta, S. Suzuki, M. Inamitsu, K. Yamamoto, Periostin in allergic inflammation, Allergol. Int. 63 (2014) 143–151.
[2] W. Li, P. Gao, Y. Zhi, W. Xu, Y. Wu, J. Yin, et al., Periostin: its role in asthma and its potential as a diagnostic or therapeutic target, Respir. Res. 16 (2015) 57.
[3] S. Takeshita, R. Kikuno, K. Tezuka, E. Amann, Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I, Biochem. J. 294 (Pt 1) (1993) 271–278.
[4] L. Gillan, D. Matei, D.A. Fishman, C.S. Gerbin, B.Y. Karlan, D.D. Chang, Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility, Cancer Res. 62 (2002) 5358–5364.
[5] C.J. Kim, T. Isono, Y. Tambe, T. Chano, H. Okabe, Y. Okada, et al., Role of alternative splicing of periostin in human bladder carcinogenesis, Int. J. Oncol. 32 (2008) 161–169.
[6] S.J. Conway, K. Izuhara, Y. Kudo, J. Litvin, R. Markwald, G. Ouyang, et al., The role of periostin in tissue remodeling across health and disease, Cell. Mol. Life Sci. 71 (2014) 1279–1288.
[7] A.D. Parulekar, M.A. Atik, N.A. Hanania, Periostin, a novel biomarker of TH2-driven asthma, Curr. Opin. Pulm. Med. 20 (2014) 60–65.
[8] P.G. Woodruff, H.A. Boushey, G.M. Dolganov, C.S. Barker, Y.H. Yang, S. Donnelly, et al., Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 15858–15863.
[9] M.C. Peters, Z.K. Mekonnen, S. Yuan, N.R. Bhakta, P.G. Woodruff, J.V. Fahy, Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma, J. Allergy Clin. Immunol. 133 (2014) 388–394.
[10] G. Jia, R.W. Erickson, D.F. Choy, S. Mosesova, L.C. Wu, O.D. Solberg, et al., Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients, J. Allergy Clin. Immunol. 130 (2012) 647–654 (e10).
[11] H. Matsumoto, Serum periostin: a novel biomarker for asthma management, Allergol. Int. 63 (2014) 153–160.
[12] J.R. Arron, D.F. Choy, H. Scheerens, J.G. Matthews, Noninvasive biomarkers that predict treatment benefit from biologic therapies in asthma, Ann. Am. Thorac. Soc. 10 (Suppl) (2013) S206–S213.
[13] C.E. Brightling, P. Chanez, R. Leigh, P.M. O'Byrne, S. Korn, D. She, et al., Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial, Lancet. Respir. Med. 3 (2015) 692–701.
[14] W.W. Busse, M. Wang, J. Gibson, M. Gottlow, M. Braddock, G. Colice, TROPOS: designing a clinical trial to evaluate the oral corticosteroid-sparing effect of a biologic in severe asthma, Clin. Invest. (Lond) 5 (2015) 723–730.
[15] R.A. Panettieri Jr., C. Brightling, U. Sjobring, A. Péterffy, G. Tornling, S.Z. Daoud, et al., STRATOS 1 and 2: considerations in clinical trial design for a fully human monoclonal antibody in severe asthma, Clin. Invest. (Lond) 5 (2015) 701–711.
[16] K. St Ledger, S.J. Agee, M.T. Kasaian, S.B. Forlow, B.L. Durn, J. Minyard, et al., Analytical validation of a highly sensitive microparticle-based immunoassay for the quantitation of IL-13 in human serum using the Erenna immunoassay system, J. Immunol. Methods 350 (2009) 161–170.
[17] J. Corren, R.F. Lemanske, N.A. Hanania, P.E. Korenblat, M.V. Parsey, J.R. Arron, et al., Lebrikizumab treatment in adults with asthma, N. Engl. J. Med. 365 (2011) 1088–1098.
[18] N.A. Hanania, M. Noonan, J. Corren, P. Korenblat, Y. Zheng, S.K. Fischer, et al., Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies, Thorax 70 (2015) 748–756.
[19] N. Jeanblanc, S. Manetz, R. Lee, M. Liang, P. Choudhury, R. Varkey, et al., A new investigational use only automated immunoassay for periostin, Eur. Respir. J. 46 (2015), http://dx.doi.org/10.1183/13993003.congress-2015.PA3936.
[20] P.M. Hemken, N. Jeanblanc, M. Datwyler, S. Brophy, S. Manetz, R. Lee, et al., Periostin investigational use only automated immunoassay for Abbott Architect®, J. Thorac. Oncol. (2015) S407–S760.
[21] F.A. Quinn, Architect® i2000® and i2000$_{SR}$® Analyzers, in: D. Wild (Ed.), The Immunoassay Handbook, 3rd ed., Elsevier Ltd, Amsterdam, Netherlands, 2005, pp. 406–411.
[22] M. Castro, S.E. Wenzel, E.R. Bleecker, E. Pizzichini, P. Kuna, W.W. Busse, et al., Benralizumab, an *anti*-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study, Lancet. Respir. Med. 2 (2014) 879–890.
[23] Clinical and Laboratory Standards Institute (CLSI), Evaluation of precision performance of quantitative measurement methods; approved guideline, CLSI Document EP05-A2, 2nd ed., CLSI, Wayne, PA, 2004.
[24] Clinical and Laboratory Standards Institute (CLSI), Evaluation of the linearity of quantitative measurement procedures: a statistical approach; approved guideline, CLSI Document EP06-A, CLSI, Wayne, PA, 2003.
[25] N.D. Christofides, Free Analyte Immunoassay, in: D. Wild (Ed.), The Immunoassay Handbook, 2nd ed., Nature Publishing Group, London, UK, 2001, pp. 61–77.
[26] Clinical and Laboratory Standards Institute (CLSI), Interference testing in clinical chemistry; approved guideline, CLSI Document EP07-A2, 2nd ed., CLSI, Wayne, PA, 2005.
[27] Clinical and Laboratory Standards Institute (CLSI), Evaluation of detection capability for clinical laboratory measurement procedures; approved guideline, CLSI Document EP17-A2, 2nd ed., CLSI, Wayne, PA, 2012.
[28] S. Hoersch, M.A. Andrade-Navarro, Periostin shows increased evolutionary plasticity in its alternatively spliced region, BMC Evol. Biol. 10 (2010) 30.
[29] L. Morra, M. Rechsteiner, S. Casagrande, A. von Teichman, P. Schraml, H. Moch, et al., Characterization of periostin isoform pattern in non-small cell lung cancer, Lung Cancer 76 (2012) 183–190.
[30] J. Tate, G. Ward, Interferences in immunoassay, Clin. Biochem. Rev. 25 (2004) 105–120.
[31] M. Matsusaka, H. Kabata, K. Fukunaga, Y. Suzuki, K. Masaki, T. Mochimaru, et al., Phenotype of asthma related with high serum periostin levels, Allergol. Int. 64 (2015) 175–180.
[32] J.R. Arron, C.T. Holweg, C. Eng, S. Salazar, L.A. Roth, M.A. Seibold, et al., Range and distribution of type 2 inflammatory biomarkers in children and adolescents with and without asthma, Am. J. Respir. Crit. Care Med. A3846-A (2014).
[33] Y. Inoue, K. Izuhara, S. Ohta, J. Ono, N. Shimojo, No increase in the serum periostin level is detected in elementary school-age children with allergic diseases, Allergol. Int. 64 (2015) 289–290.